ANSWERING REVIEWERS

January 21, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7395-review.doc).

Title: Mesenchymal stem cells for treatment of aortic aneurysms.
A topic highlight of the literature

Author: Aika Yamawaki-Ogata, Ryotaro Hashizume, Xianming Fu, Akihiko Usui, Yuji Narita

Name of Journal: *World Journal of Stem Cells*

ESPS Manuscript NO: 7395

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

General comments:
We appreciate the thoughtful and constructive comments provided by the reviewers and respond to their recommendations and concerns below. The parts of the manuscript that have been significantly revised are highlighted in red.

(1) Reviewer 1
-Comment 1
In this review, the authors summarize previous research results on using MSC for aortic aneurysm, focused on two studies from their lab (Hashizume et al, Fu et al) but also considering other related studies. It is a good review of AA and considerations for how likely paracrine effects impact the disease. While MSC biology is briefly mentioned, there is no discussion of MSC phenotype, or the inherent heterogeneity of the cell sources used in various studies. Since the review is for a stem cell audience, the authors should add a section detailing what exactly they are calling MSCs (are they really stem cells?), the different phenotypes used in the field as MSCs, and how this might influence effects noted in various studies. MSC is a term that is too often used in a non-rigorous manner, so this would be a good opportunity for the authors to help clarify the issue.

-Answer 1
We appreciate the reviewer's insightful and practical comment on this point. We have added to the part of the manuscript where the discussion describes human MSC phenotype (1st paragraph of the **DETERMINATION OF MESENCHYMAL STEM CELLS)** and MSC phenotype that were used in AA experimental studies (7th paragraph of the **TREATMENT OF AORTIC ANEURYSMS USING MESENCHYMAL STEM CELLS**). We have also summarized these reports in the new table 1.

-Changes 1
The 1st paragraph of the **DETERMINATION OF MESENCHYMAL STEM CELLS** has been revised as follows:
"Early in culture, the spindle-shaped plastic-adherent cells do not appear uniformly by contamination

of hematopoietic cells, but this heterogeneity gradually decreases influenced by culture conditions and consecutive passages [36,37]. The International Society of Cell Therapy (ISCT) criteria propose that human MSC should be positive for the expression of CD73, CD90 and CD105 (≥ 95% positive), and lack expression of CD34, CD45, CD11b or CD14, CD19 or CD79α, and HLA-DR (≤ 2% positive). Also, MSCs should differentiate into osteogenic, adipogenic and chondrogenic lineage (Table 1)[38]. However, CD73 and CD105 are also expressed on fibroblast and endothelial lineage cells and CD90 is also expressed on haematopoietic stem cells[39,40]. To improve purity of the human MSC population, several studies have been performed using a combination such as Stro-1, CD271, CD146 and PDGFR-α, not only CD73, CD90 or CD105[41-43].

We have also added the last part of the **TREATMENT OF AORTIC ANEURYSMS USING MESENCHYMAL STEM CELLS** as follows:

" In these studies, MSC phenotypic characteristics have been identified by surface marker and pluripotency. Although these different positive and negative immunophenotypes are concerned with differences in animal species, they resemble human MSC immunophenotypic characteristics (Table 1)."

In addition, the new table 1 is presented as follows:

Table 1 MSC phenotypic characteristics

		Positive marker	Negative marker	Pluripotency	Ref.
ISCT criteria	Human MSC	CD73, CD90, CD105	CD34, CD45, CD11b or CD14, CD19 or CD79α, HLA-DR	Osteogenic Chondrogenic Adipogenic	38
In AA experimental studies	Mouse BM-MSC	CD44, CD106, Sca-1	CD11b, CD31, CD34, CD45, CD86, CD117	Osteogenic Chondrogenic Adipogenic	51, 53
	Human placental-MSC	CD29, CD44, CD73, CD90, CD105	CD14, CD19, CD34, CD45, HLA-DR	Data not shown	54
	Rat BM-MSC	CD44, CD73, CD90, CD105	CD11b, CD45	Data not shown	56
	Pig ASC	CD73, CD90, CD105	CD14, CD11b	Osteogenic Chondrogenic Adipogenic	57
	Pig BM-MSC	CD13, CD29	CD31, CD34, CD45	Data not shown	58

(2) Reviewer 2

-Comment 1

REF: ESPS Manuscript No.: 7395 The manuscript Mesenchymal stem cells for treatment of aortic aneurysm, by Yamawaki-Ogata et al. is e review on current treatments against aortic aneurysm, with particular emphasis on a cell therapy approach based on mesenchymal stem cells. The manuscript is very interesting, the cited works are relevant and the references are updated. In addition, the content falls certainly within the scope of the journal. However, I have a major concern regarding the general structure of the manuscript. While it is submitted in form of review, I found it hard to recognize it as such. The author should do their best to avoid any ambiguity, since often the style and the way data are presented make hard to read the paper as a review. In particular, the fact that so many figures are shown, and that the text stile comments them as if they were original, is very confounding. The authors should clearly state in the text, figure legends and anywhere appropriate where the data are taken from

- by the way, since several figures are extremely similar to previously published material I do not know how they should deal with reproduction permissions and copyright.

-Answer 1
We appreciate the reviewer's concerns on these points. Of course, this paper does not have the usual structure of a review paper, however, there was just seven papers including our two describing MSC treatment for aortic aneurysm. It is very promising approach for life-threatening disease. So we presented summary and comparison of very few papers. We think this paper was a mini-review regarding MSC treatment for AA. Therefore, we have deleted the previous figures and have re-edited with 3 new figures.

Three new figures and figure legends are presented as follows:

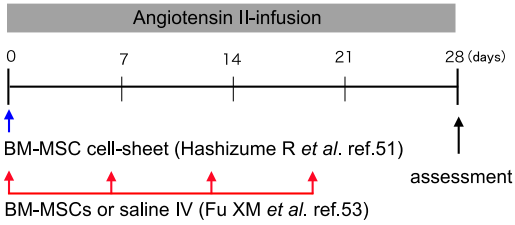


Fig. 1

Figure 1 Diagram of BM-MSC cell-sheet implantation or BM-MSC IV-administration protocol. At the time of Alzet osmotic minipump implantation, the BM-MSC cell-sheet was implanted into the nearby abdominal aortic adventitia[51], and 1 × 10^6 BM-MSCs (in 0.2 mL saline) or 0.2 mL saline were injected intravenously via the tail vein every 4 weeks[53]. Mice were sacrificed and assessed on day 28.

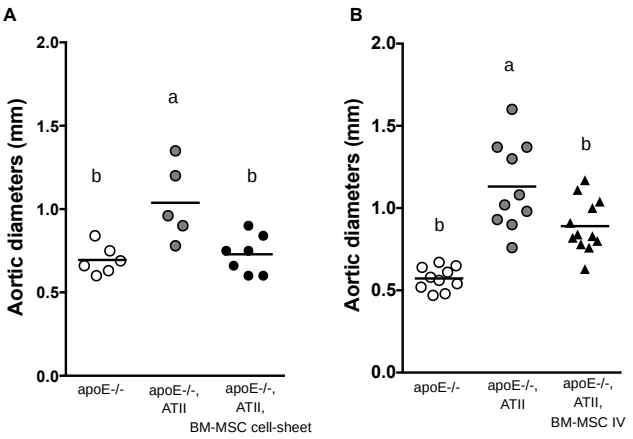


Fig. 2

Figure 2 BM-MSC cell-sheet implantation or BM-MSC IV-administration attenuates aortic aneurysm progression and expansion. Aortic diameter was measured at the infrarenal aorta in the BM-MSC cell-sheet (A) or the BM-MSC IV-administration (B). Data are assessed by one-way ANOVA with Bonferroni correction. [a] $P < 0.05$ vs apoE$^{-/-}$ group, [b] $P < 0.05$ vs apoE$^{-/-}$ + ATII group. Data are from Hashizume R *et al*[51] and Fu XM *et al*[53].

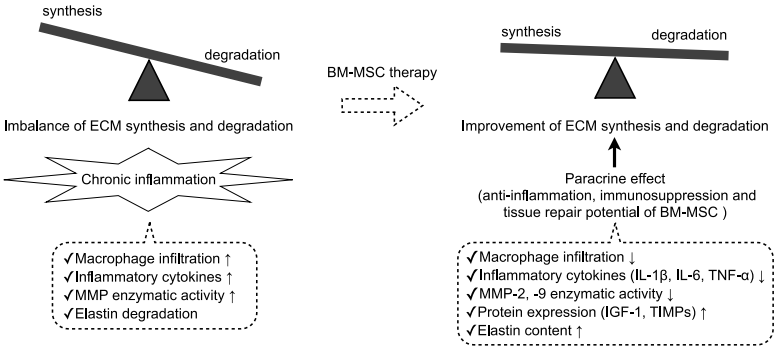


Fig. 3

Figure 3 Attenuation of aortic aneurysm development and growth is associated with improvement of the imbalance between degradation and synthesis of ECMs by BM-MSC therapy.

-Comment 2

This, being said, the English would need revision by a mother tongue editor and the overall length of the manuscript should be verified.

-Answer 2

We have re-checked our manuscript carefully and the manuscript has been revised by a native English editor.

-Comment 3

In addition, some of the cited methodological approaches should be better described, since they might be of great interest/critical relevance (injection site, delivery system etc.)

-Answer 3

In line with the reviewer's comment, we added the text of methodology on injection site and delivery system in the 1st paragraph of the **FUTURE PERSPECTIVE OF MESENCHYMAL STEM CELL THERAPY FOR AORTIC ANEURYSMS.** We also summarized these reports in the new table 3.

-Changes 3

"Second, the delivery methods of MSC in these studies are respectively different. Investigators have performed administration using several methods involving implantation of cell-sheet, IV-administration, direct injection into aortic wall, and catheter delivery (Table 2). Among them, although IV-administration is the least invasive and simple procedure, the targeting ability is lower and injected MSCs are trapped in other tissues such as lung, spleen, liver and kidney. In contrast, the implantation of cell-sheet and the direct injection into aortic wall make it possible to target AA. However, these are relatively invasive procedures. On the other hand, endovascular delivery using a catheter is less invasive and has a high targeting ability."

In addition, new table 3 is presented as follows:

Table 3 Methodology of delivery system

Delivery system	Administration site	Localization, timing	Delivery system		Ref.
			Merits	Demerits	
Cell-sheet	Adventitia of abdominal aorta	Adventitia, 4 weeks after implantation	High targeting ability	Invasive procedure by laparotomy	51
IV	Tail vein	Media and/or adventitia, At 4 weeks	Least invasive	Low targeting ability and trapping in other tissue	53
IV	Tail vein	Data not shown	Least invasive	Low targeting ability and trapping in other tissue	54

Catheter	Clamped endovascular	Intima 1 week after injection	Less invasive and high targeting ability	Requirement of a surgical procedure or advanced catheter intervention	56
Catheter	Clamped endovascular	Media 3 weeks after injection	Less invasive and high targeting ability	Requirement of a surgical procedure or advanced catheter intervention	57
Direct injection	Injured aortic wall	Aortic wall, 1 week after injection	High targeting ability	Risk of rupture	58

-Comment 4

A positive remark concerns a specific statement,i i.e. the importance of cellular activities in the treatment of AA. I find the major point presented by the authors very convincing: the fact that cellular treatment can be of the greatest efficacy (possibly through an indirect effect on the ECM). To support this notion, I would cite an article by Galmiche et al. (Circ Res. 2013 Mar 29;112(7):1035-45), who found that inactivation of serum response factor contributes to decrease vascular muscular tone and arterial stiffness in mice; in this experimental model SRF was specifically inactivated in smooth muscle cells; thus, an intracellular sensor of cell stress and transcription factor controls vasomotor tone and cell-matrix attachment affecting arterial elasticity in large arteries.

-Answer 4

We appreciate the reviewer's insightful comment on this point. We have added to the part of the manuscript where the discussion describes cellular activities in the treatment of AA (3rd paragraph of the **FUTURE PERSPECTIVE OF MESENCHYMAL STEM CELL THERAPY FOR AORTIC ANEURYSMS**), and the article by Galmiche *et al*. (Circ Res. 2013 Mar 29;112(7):1035-45) have cited as ref.61.

-Changes 4

The 3rd paragraph of the **FUTURE PERSPECTIVE OF MESENCHYMAL STEM CELL THERAPY FOR AORTIC ANEURYSMS** has been revised as follows:

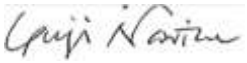
"TGF-β is an important signal that induces SMC differentiation and increases serum response factor (SRF) expression through an increase in transcription of the SRF gene[60]. Moreover, SRF controls vasoconstriction via SMC phenotypic modulation[61]. This fact might be supported by cellular activities in the treatment of AA using MSC therapy."

3 References and typesetting were corrected as described above, and the changed parts are highlighted in red.

4 We also added subtitles in each paragraph and the abbreviations list after figure legends. In addition, manuscript title has been revised from "Mesenchymal stem cells for treatment of aortic aneurysm" to "Mesenchymal stem cells for treatment of aortic aneurysms"

Thank you again for reviewing our manuscript and we hope it is now suitable for publishing in the *World Journal of Stem Cells*.

Sincerely yours,



Yuji Narita, MD, PhD.
Associate Professor and Principal Investigator
Department of Cardiac Surgery,
Nagoya University School of Medicine, 65 Tsurumai, Showa, Nagoya 466-8550, Japan
Phone: +81-52-744-2376

Fax: +81-52-744-2383
E-mail: ynarita@med.nagoya-u.ac.jp